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## SPECIFICATION

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## COMPLETE SPECIFICATION

## Time-Temperature Indicator

GT. BRIT.  
DIV.

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We, HYGRADE FOOD PRODUCTS CORPORATION, a corporation organized under the laws of the State of New York, United States of America, of 2811 Michigan Avenue, Detroit, State of Michigan, United States of America, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a method of determining the extent of microbial spoilage of foodstuffs, such as meat, and to an indicator, particularly a time-temperature indicator, for determining the extent of such spoilage.

Storage of meats takes place under refrigerated conditions to prevent microbial spoilage from occurring. However, it frequently happens that meat products in the course of handling are subjected to elevated temperatures at which rapid growth of food spoiling microorganisms takes place. Casual inspection of the food product is not sufficient to detect the extent of spoilage during the inductive phase, and for this reason products may appear sound to within an hour of the time that frank spoilage is manifest. It is apparent from the handling of meat products that there is lacking a simple inexpensive way of designating when the particular food item has been subjected to adverse conditions for an undue period. Prior art workers have been investigating this problem for some time but thus far no one has suggested a simple solution. Following extensive investigation, it was found that a simple and economic solution to the problem does exist.

Accordingly, it is an object of this invention to describe an indicator which is activated by relationships of time and temperature which are in all respects similar to those conducive to microbial spoilage. The indicator can be regarded as self-integrating with respect to time and temperature. Response to time and temperature follows the same pattern as the organisms responsible for microbial spoilage.

In accordance with the invention there is

[Price 4s. 6d.]

provided a method of determining the extent of microbial spoilage of foodstuffs such as meat, which comprises utilizing a micro-organism capable of accelerated growth with increasing temperature, initiating the growth of said microorganism at a desired time in a nutrient medium in the same environmental conditions as the foodstuffs, and providing an indicator system for designating when the growth of said microorganism exceeds a predetermined desired limit and a time-temperature indicator for determining the extent of such spoilage, which comprises a package containing a microorganism capable of accelerated growth with increasing temperatures, and also containing ingredients for a nutrient medium, means for initiating at a desired time the growth of said microorganism in the nutrient medium, and an indicator system for designating when the growth of said microorganism exceeds a predetermined value.

The microbial spoilage indicator of this invention comprises a population of viable microorganisms, which grow in approximately the same temperature range as microorganisms associated with spoilage, a nutrient or growth medium which may contain a buffer, and an indicator which will show when the microbial population has passed a predetermined point. In the active indicator all of the named components are in a common aqueous system. While it is possible to maintain the system frozen up to the time of use, it is more feasible to maintain the water apart from the microorganisms and nutrient until the time of use. In the practice of this invention the microorganisms, nutrient, indicator, and water are maintained separate and apart or out of contact with the meat product to avoid problems of contamination. For this purpose these materials are kept within a separate package which may take the form of a transparent plastic bag. The structural features of the package will be described in greater detail below.

The microbial species employed is preferably one which is not capable of inducing

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food poisoning, i.e., it is non-pathogenic. Even though the microorganism will not be in contact with the meat product, it is advisable to use a non-pathogenic microorganism because of the possibility, through mischance, that such an organism will contaminate the food product. The type of organism which can be used for this invention may vary considerably and include a large number of classes. The important feature in the selection of a microorganism is that it must be capable of growth over the temperature range conducive to spoilage. In this regard the microorganism may be any acid producing bacteria which undergoes rapid growth over a temperature range of about 45° to about 100° F. when in contact with a suitable growth medium or nutrient. As a result of growth, the acid developed increases in concentration to the point that it will affect an acid-base indicator and cause a color change therein. The change in the color of the indicator will serve to alert the handler of the meat product that the danger of frank spoilage is imminent. A microorganism which can be used in this instance is the homo-fermentative lactic acid bacteria such as, for example, *Lactobacillus lactis*, *Lactobacillus helveticus*, *Lactobacillus acidophilus*, *Lactobacillus bifidus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus plantarum*. The microorganism can also be a mold with colored spores or one which undergoes extensive mycelial growth. Bacteria or yeast growth will cause turbidity, and an intensity scale calibrated to show the appropriate turbidity at excessive growth can be used as an indicator.

In general, the quantity of microorganism relative to the nutrient can vary over a wide range. In each specific case the population of microorganisms per unit of nutrient is predetermined to produce a change within a temperature range which is expected to be experienced under actual service conditions. In the case of the homofermentative bacteria, it is convenient to us 500 to 20,000 viable cells per 1 ml. of dissolved medium.

The nutrient to be used is selected on the basis of being one which will promote growth of microorganisms within the temperature range to be experienced in the course of handling the meat product. The growth medium must provide a nitrogen source such as, for example, the peptones which the partially hydrolyzed natural proteins, e.g. bacto-peptone. Other examples of nitrogen supplying growth medium are bacto-tryptone, proteose peptone, bacto-tryptose, neopeptone, bacto-casitone, bacto-protone, and bacto-casamino acids. Various other amino acids can also be used. The growth medium must also include a carbon source for the microorganisms. The carbon containing or supplying material can include the monosaccharides, such as glucose, levulose, mannose or galactose;

disaccharides, such as sucrose, lactose or maltose; the polysaccharides, including starch, inulin, dextrin and glycogen; the alcohols, including glycerol, adonitol, mannitol, dulcitol and sorbitol; the glucosides, including salacin and amygdalin; the noncarbohydrate compounds, including inositol. The quantity of growth medium which is present with the microorganism is calculated to produce a rapid growth within the specified temperature range so that the change affected by reference to the indicator will serve as a designation to the handler of the product that the food product has undergone a more than safe limit of subjection to elevated temperature. It should be borne in mind that the quantity of nutrient is correlated with the population of microorganism and the type of indicator to be used.

The indicator is selected on the basis of the type of microorganism and nutrient which are used in the system. Where the microorganisms are acid-producing bacteria, an acid-base indicator is employed. In this connection the indicator is, for example, bromophenol blue, bromochlorophenol blue, bromocresol green, chlorocresol green, methyl red, chlorophenol red, bromophenol red, bromocresol purple, bromothymol blue and phenol red. The indicator must not exceed a significant inhibitory effect on the activity of the microbial culture. It will be noted from the type of indicators enumerated above that the color change will take place at various pH levels. This means that the indicator must be correlated with the type of microorganism used and the nutrient to be supplied therefor. When yeast is used as the microorganism, the indicator is a turbidity scale which shows the extent of colony growth representing the point at which the temperature history of the food product has been undesirable from the standpoint of spoilage.

The indicator system of this invention is preferably maintained in the anhydrous state until the start of the period it is to monitor. Generally the indicator system is not activated until the meat is packaged or removed from inventory. The indicator system is activated by bringing all components (microorganism, nutrient and indicator) into a common aqueous solution. In order to assure uniformity of activity it is desirable to utilize lyophilized or freeze dried cultures. The growth medium or nutrient is likewise maintained in a dried state. In this way the indicator can be put into operation at the convenience of the handler in order that he can control zero time in respect to the history of the meat product. The indicator system is placed in a suitable package. The package can be a sealed envelope divided into two compartments which are separated by a rupturable membrane. The material making up the package is either translucent or transparent so that the color

change can be witnessed without difficulty. Various plastic materials lend themselves to use for this purpose such as, for example, polyethylene, Mylar, Kel-F, Teflon, lucite (Mylar and Teflon being Registered Trade Marks). In any case, the bag or package is preferably flexible so that the separated membrane can be easily ruptured by the operator and put the indicator into operation. As might

be expected, the package or envelope is water-proof so that the only source of water is that contained within the separate compartment of the package.

In order to better understand the present invention, reference will be had to specific examples. The following are specific illustrations of compositions which can be used for the purpose of this invention:

## EXAMPLE I

Percentage	Name of Material
1%	Lyophilized <i>Pediococcus cerevisiae</i>
1%	Peptone
1%	Yeast extract
1%	Dextrose
0.01%	Bromocresol purple
Balance	Water

## EXAMPLE II

Percentage	Name of Material
1%	Lyophilized <i>Pediococcus cerevisiae</i>
1%	Tryptone
0.5%	Dextrose
0.004%	Bromocresol purple
Balance	Water

## EXAMPLE III

Percentage	Name of Material
0.25%	Lyophilized <i>Pediococcus cerevisiae</i>
0.5%	Yeast extract
1.5%	Dextrose
0.25%	Potassium monohydrogen phosphate
0.002%	Bromothymol blue
Balance	Water

## EXAMPLE IV

Percentage	Name of Material
0.50%	Active dry yeast
0.4%	Yeast extract
0.1%	K H <sub>2</sub> PO <sub>4</sub>
1.0%	Sucrose
98%	Water

(In this case, gas production as manifest by swelling of the package indicates danger of spoilage.)

## EXAMPLE V

Percentage	Name of Material
0.2%	Dry bread inoculated with spores from <i>Penicillium</i> (mold species)
2.0%	Sucrose
0.2%	Potassium nitrate
0.1%	Potassium monohydrogen phosphate
0.1%	Magnesium sulphate
0.1%	Sodium chloride
0.01%	Ferrous sulphate
Balance	Water

(At the danger point, this indicator becomes a green mat of mold.)

Having thus provided a description of the invention along with specific examples thereof, it should be understood that no undue restrictions or limitations are to be imposed by reason thereof but that the present invention is defined by the appended claims.

## WHAT WE CLAIM IS:—

1. A method of determining the extent of microbial spoilage of foodstuffs such as meat, utilizing a microorganism capable of accelerated growth with increasing temperature, which comprises initiating the growth of said microorganism at a desired time in a nutrient medium in the same environmental conditions as the foodstuffs, and providing an indicator system for indicating that the growth of said

microorganism has exceeded a predetermined desired limit.

2. A time-temperature indicator for determining the extent of microbial spoilage of foodstuffs such as meat, when placed in the same environmental conditions, which comprises a package containing a microorganism capable of accelerated growth with increasing temperatures, dry ingredients for a nutrient medium, and an indicator system for designating when the growth of said microorganism exceeds a predetermined value, said package being constructed to permit admission of a liquid which will initiate at a desired time the growth of said microorganism.

3. An indicator according to claim 2, in

- which said package is constructed to have two compartments separated by a membrane, one compartment containing said microorganism, a dry nutrient, and said indicator system, and the other compartment containing said liquid comprising water, said membrane being adapted to be broken manually to form the nutrient medium and contact said microorganism therewith.
- 5 4. An indicator according to claim 2 or 3, in which the microorganism is lyophilized.
- 10 5. An indicator according to any one of claims 2 to 4, in which the organism is a culture of *Pediococcus cerevisiae*.
- 15 6. An indicator according to any one of claims 2 to 4, in which said microorganism is an acid producing microorganism.
7. An indicator according to claim 6, in which the microorganism is a lactic acid producing bacteria.
- 20 8. An indicator according to any one of claims 2 to 7, in which the indicator system comprises an acid-base indicator.
9. An indicator according to any one of claims 2 to 8, in which the nutrient comprises peptone, yeast extract, and dextrose.
- 25 10. A time-temperature indicator for determining the extent of microbial spoilage of foodstuffs such as meat when placed in the same environmental conditions, substantially as hereinbefore described.
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